REMARKS/ARGUMENTS

Claims 9-18 are cancelled; claims 1-4 are amended; and claims 19-26 are newly added.

Support for Amendments

Amendments to the claims are supported throughout the application including the specification and claims as originally filed and contain no new matter. Specific support is indicated for each amendment for the convenience of the examiner.

Claim 1 is amended to newly recite that said first and said second recombination sequences are different and form a complementary set of recombination sequences enabling said constructs to recombine with each other. The application as filed demonstrates first and second recombination sequences that are different in the examples. For instance, page 18 shows a 5' first recombination sequence including a 250 bp sequence and page 19 shows a 3' second recombination sequence including a 208 bp sequence. In addition, page 19-20 shows a 5' second recombination sequence including a 208 bp sequence and page 20 shows a 3' first recombination sequence including a 250 bp sequence. Thus, the recombination sequences are different.

Support for recombination between the constructs may be found at page 3, line 32 through page 4, line 3, which provides,

> "The skilled addressee will appreciate that once the said at least two heterologous constructs are transformed into the moss plant cell… they will undergo recombination with each other many times over."

Further support is found at page 4, lines 19-22, which provides in part, "The at least first and the at least second recombination sequences form a complementary set that make it possible for the constructs of the invention to recombine with each other."

Claims 2-4 are amended to correct claim grammar.

Claim 19 is newly added. Claim 19 provides,

A set of nucleic acid vectors suitable for amplifying gene expression in a moss plant cell, wherein said set of nucleic acid vectors comprises:

1) at least a first heterologous nucleic acid construct comprising at least one heterologous nucleotide sequence operably linked to a promoter, wherein said construct is flanked at the 5' end thereof by a first recombination sequence and is flanked at the 3' end of said construct by a second recombination sequence in the same orientation as the first; and

2) at least a second heterologous nucleic acid construct comprising at least one heterologous nucleotide sequence operably linked to a promoter, wherein said construct is flanked at the 5' end thereof by said second recombination sequence and is flanked at the 3' end of said construct by said first recombination sequence in the same orientation as the second;

wherein said first and said second recombination sequences are different and form a complementary set of recombination sequences enabling said vectors to recombine with each other.

Support may be found in claim 1 as amended, which describes methods of amplifying gene expression using the at least two heterologous nucleic acid constructs as provided in claim 19. Further support is provided in claims 9 and 10 as filed, which are directed towards heterologous DNA constructs. Still further support may be found within the definitions of "vector" and "expression vector" provided on pages 7 and 8.

New claims 20-24 correspond to previous claims 12-16, rewritten to depend from new independent claim 19.

Claim 25 is newly added and corresponds to previous claim 17 rewritten to depend from new claim 19. Claim 25 also clarifies "use" includes the steps of providing moss protenema cells transformed with the set of DNA vectors according to claim 19 and inducing expression of protein encoded in the DNA constructs. Further support may be found at page 12, lines 7- 12, which provide,

> "The present invention also provides methods comprising the introduction of such constructs comprising appropriate heterologous sequences into a moss plant cell and/or induction of expression of a construct of the invention within a moss plant cell, by application of a suitable stimulus e.g. an effective exogenous inducer."

Claim 26 is newly added, depends from claim 25 and adds that the moss protonema cells are *Physcomitrella patens.* Support may be found at page 12, lines 12-14, which provide in part,

> "Suitable moss plant cells include the moss protoplast, and cells comprised in the protonema, such as those derived from *Physcomitrella patens*."

Response to Rejections Under 35 USC 112, 2nd paragraph

I. Standard for definiteness

The definiteness of language employed must be analyzed not in a vacuum, but in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing ordinary skill in the art. Allen Archery Inc. v. Browning Mfg. Co., 2 USPQ2d 1490, 1494 (Fed. Cir. 1987). "If the claims, read in light of the specification, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more." North Am. Vaccine, Inc. v. American Cyanimid Co., 28 USPQ2d 1333, 1339 (Fed. Cir. 1993).

II. Rejection of Claims 17 and 18 as being indefinite

The examiner rejects claims 17 and 18 as being indefinite due to the lack of specific

method steps in the "use" claims.

Claims 17 and 18 are cancelled. New "use" claims 25 and 26, which generally correspond to previous claims 17 and 18 recite specific steps involved in the use of the nucleic acid vectors. Accordingly, applicants respectfully request the rejection be withdrawn.

III. Rejection of claims 4, 15 and 18 as being vague and indefinite over the recitation of "derived from"

The examiner rejects claims 4, 15 and 18 due to the recitation of "derived from" as this is allegedly non-specific and relative in nature.

Claim 4 is amended to remove the terminology "derived from" in favor of "selected from the group consisting of" to clarify the meaning of claim 4.

Claims 15 and 18 are cancelled; however, claims 23 and 26 generally correspond to previous claims 15 and 18. Neither claims 23 nor 26 recite "derived from".

Accordingly, applicants respectfully request the rejections be withdrawn.

Introduction to the Invention

The present invention provides methods and systems for amplifying gene expression in a transformed moss plant cell. Broadly, the invention includes two constructs, each having at least one heterologous nucleotide sequence. The first construct is flanked at the 5' end by a first recombination sequence and is flanked at the 3' end by a second recombination sequence in the same orientation as the first. The second construct is flanked at the 5' end by the second recombination sequence and is flanked at the 3' end by the first recombination sequence in the same orientation as the second. The first and second recombination sequences are different and form a complementary set of recombination sequences, which enable the constructs to recombine with each other. The inventors surprising found such a system results in an increase in the integrated copy number of heterologous nucleic acid constructs in regenerated tissue which in turn correlated with an increases protein expression levels.

Response to Rejection Under 35 U.S.C. §102 (Anticipation)

Claims 9-10 are cancelled; however, for completeness new claim 19 generally corresponds to claims 9 and 10; and thus claim 19 is not anticipated by Gilbertson et al

The examiner rejects claims 9 and 10 under 35 U.S.C. § 102(b) as being anticipated by Gibertson et al. Although claims 9-10 are cancelled, claim 19 is newly added and is directed towards a set of nucleic acid vectors suitable for amplification of gene expression in a moss plant cell. Previous claims 9 and 10 were directed towards nucleic acid constructs. Thus, for completeness the rejection is addressed in comparison to new claim 19 and claims that depend therefrom.

Anticipation requires a single prior art reference disclose each and every element of the claim under consideration." W.L. Gore & Assocs. V. Garlock, Inc., 220 USPQ 303, 313 (Fed. Cir.1983). However, it is not enough that the reference discloses all of the claimed elements in isolation. Rather, as stated by the Federal Circuit, the prior art reference must disclose each and every element of the claimed invention "arranged as in the claim." Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co., 730 F.2d 1452, 221 USPQ 481, 485 (Fed. Cir.1984). Further, the prior art must be such that a person of ordinary skill in the art would consider there to be no difference between the claimed invention and the referenced disclosure. In re Gurley, 27 F.3d 551, 31 USPQ2d 1130, 1132 (Fed Cir. 1994).

The examiner argues Gilbertson et al disclose a DNA construct comprising in 5' to 3' direction, a recombination site, a promoter operatively linked to a heterologous gene, and a second recombination site in the same orientation as the first.

In contrast to Gilbertson et al. claim 19 includes a set of nucleic acid vectors suitable for amplifying gene expression in a moss plant cell, wherein said set of nucleic acid vectors comprises: 1) at least a first heterologous nucleic acid construct comprising at least one heterologous nucleotide sequence operably linked to a promoter, wherein said construct is flanked at the 5' end thereof by a first recombination sequence and is flanked at the 3' end of said construct by a second recombination sequence in the same orientation as the first; and 2) at least

a second heterologous nucleic acid construct comprising at least one heterologous nucleotide sequence operably linked to a promoter, wherein said construct is flanked at the 5' end thereof by said second recombination sequence and is flanked at the 3' end of said construct by said first recombination sequence in the same orientation as the second; wherein said first and said second recombination sequences are different and form a complementary set of recombination sequences enabling said vectors to recombine with each other. Gilbertson et al. do not teach this combination of elements and do not teach the combination as arranged in the claim.

A. In Gilbertson et al. homologous recombination occurs between the construct and the plastid DNA and not between a set of vectors.

Claim 19 includes the limitation that the first and second recombination sequences are different and form a complementary set of recombination sequences enabling the vectors to recombine with each other. Thus, in the present invention recombination occurs between the vectors themselves.

In contrast, Gilbertson et al. teach homologous recombination between a construct and plastid DNA. This is summarized in the abstract, which states in part, "A method for improved plastid transformation efficiency via homologous recombination and nucleic acid sequences useful therefore is provided." This is also provided in paragraph [0004], which states in part,

> "In one aspect of the invention, a nucleic acid sequence comprising a 5 base pair recombination sequence motif or multiple direct repeats thereof that increase the frequency of integration of a selected transgene through plastid transformation by homologous recombination is provided. The recombination sequence motif generally comprises the sequence 5'-TATTA-3', its complement 3'-TAATA -5' and imperfect repeats of the motif that are changed by one nucleotide[.]"

Thus, in Gilbertson et al. the object is to recombine the transgene from the construct into the plastid DNA and not to direct recombination between a set of vectors. It is noted that Gilbertson et al. do contemplate a second construct within the plant cell. For instance a second construct is considered in paragraph [0042], which states in part,

> "Where more than one construct is to be used in the methods, the constructs can

> employ the use of the regions of homology to target the insertion of the construct into the same or a different position of the plastid genome."

However, while Gilbertson et al consider the use of more than one construct, the strategy remains to direct recombination between the construct and the plastid DNA. Specifically, the second construct targets "the same or a different region of the plastid genome."

Since Gilbertson et al. do not teach each element as arranged in the claims, Gilbertson et al. do not anticipate claims 19-16. Accordingly, applicants respectfully request the rejection be withdrawn.

B. Gilbertson et al. do not form a complementary set of recombination sequences enabling vectors to recombine as set forth in claims 19-26

In the present invention the first construct is flanked at the 5' end by a first recombination sequence and is flanked at the 3' end by a second recombination sequence in the same orientation as the first. The second construct is flanked at the 5' end by the second recombination sequence and is flanked at the 3' end by the first recombination sequence in the same orientation as the second. The sequences are different and form a set of recombination sequences that enable the vectors to recombine with each other. Although, Gilbertson et al. direct recombination between a construct and plastid DNA, it is also apparent that Gilbertson et al do not form a complementary set of recombination sequences as set forth by the present invention.

In Gilbertson et al. a 5 base pair recombination sequence motif (or multiple direct repeats thereof) is used to target insertion of a transgene into plastid DNA. More specifically, the method incorporates a recombination sequence 5'-TATTA-3' or variations thereof, which can target a particular region within the plastid DNA, namely a TATTA repeating region. The motifs used in Gilbertson et al. are discussed in paragraph [0042], which provides in part,

> "More particularly, the regions of homology comprise the recombination sequence motif of the present invention. The recombination sequence motif comprises a 5 base pair nucleic acid sequence or multiple repeats thereof (whether in tandem or interspersed with other nucleotides) that increase the frequency of integration of a transgene. The recombination sequence motif generally comprises the sequence

> 5'-TATTA-3', its complement 3'-TAATA -5', or imperfect variations of such motif differing by a nucleotide[.]"

The recombination sequence motif targets a region of the plastid DNA that includes a region of TATTA repeats. An overview of the system is demonstrated in FIG. 5 and summarized at paragraph [0012], which provides,

> "FIG. 5 is a schematic of integration of a fragment of DNA into a plastid genome by homologous recombination using a fragment engineered to contain TATTA repeats such that the repeats are integrated along with one or more transgenes[.]"

Targeting TATTA repeating regions of the plastid DNA is specifically provided in the examples. Referring to paragraph [0063] within example 1,

> "A plastid transformation vector containing a transgene is prepared comprising homologous flanking sequences capable of causing the integration of the transgene into the plastid genome wherein the homologous flanking sequence include sequences with naturally occurring repeats of the recombination sequence motif TATTA....The transgene in the vector is then capable of being targeted to the TATTA repeats in the tobacco chloroplast genome upon transformation of the vector into the tobacco chloroplast."

Thus, whereas the present invention includes complementary recombination sequences that enable recombination between vectors, which includes a first construct flanked at the 5' end by a first recombination sequence and flanked at the 3' end by a second recombination sequence, and a second construct flanked at the 5' end by the second recombination sequence and flanked at the 3' end by the first recombination sequence, and wherein the first and second recombination sequences are different; Gilbertson et al. direct homologous recombination between a construct and plastid DNA by providing flanking recombination sequences that mimic an area of repeating TATTA sequences in the plastid DNA.

Since Gilbertson et al. do not teach each element as arranged in the claims, Gilbertson et al. do not anticipate claims 19-26. Accordingly, applicant respectfully requests the rejection be withdrawn.

Response to Rejections Under 35 U.S.C. §103 (Obviousness)

Claims 1-8 are not obvious over Gilbertson et al in view of Schaefer

The examiner argues Gibertson et al. disclose a DNA construct comprising in a 5' to 3' direction a recombination site, a promoter operably linked to a heterologous gene, and a second recombination site in the same orientation as the first site. The examiner acknowledges that Gilbertson et al. do not teach that moss cells are transformed with the disclosed DNA. However, the examiner cites Schaefer as disclosing methods for transforming moss cells such as *Physcomitrella patens*, using DNA constructs comprising heterologous DNA operatively linked to a promoter and the cells are transformed with one or multiple constructs. Thus, the examiner concludes that it would be obvious to one of ordinary skill in the art to have transformed moss with constructs, such as those provided by Gilbertson, and thus argues the present invention is obvious.

A proper obviousness rejection requires consideration of the factual inquiries provided in Graham v. John Deere Co., 38 U.S. 1, 148 USPQ 459 (1966), including: 1) determining the scope and contents of the prior art; 2) ascertaining the differences between the prior art and the claims at issue; 3) resolving the level of ordinary skill in the pertinent art; and 4) considering the objective evidence of nonobviousness. Although Graham v. John Deere requires that certain factual inquiries be conducted to support a determination of the issue of obviousness, the actual determination of the issue requires an elevation in light of the findings in those inquiries as to the obviousness *of the claimed invention as a whole*, not merely the differences between the claimed invention and the prior art. Lear Siegelr, Inc. v. Aeroquip Corp., 221 USPQ 1025, 1033 (Fed. Cir. 1984). Further, the teachings of a prior art reference are underlying factual questions in the obviousness inquiry. Para-Ordnance Mfg., Inc. v. SGS Imp. Int'l, Inc. 73 F.3d 1085, 1088 (Fed. Cir. 1995).

A. **Gilbertson et al. in view of Schaefer do not teach recombination between constructs and do not teach the technical approach taken by claims 1-8, namely a complementary set of recombination sequences enabling two constructs to recombine as set forth in claims**

Claim 1 is amended to clarify the invention. Specifically, claim 1 is amended to recite that the first and second recombination sequences are different and that they form a complementary set of recombination sequences enabling the constructs to recombine with each other. Neither Gilbertson et al. nor Schaefer teach these limitations.

The amendment to claim 1 clarifies that gene amplification in the moss plant cell occurs, in part, through recombination between the constructs themselves. This is accomplished, in part, through the use of a first construct, which includes at least one heterologous nucleotide sequence, flanked at the 5' end by a first recombination sequence and flanked at the 3' end by a second recombination sequence. A second construct, which includes at least one heterologous sequence, is flanked at the 5' end by the second recombination sequence and flanked at the 3' end by the first recombination sequence. The first and second recombination sequences are different and form a complementary set of recombination sequences. The inventors have found this system results in recombination between the constructs themselves. This is summarized at page 3, line 31 through page 4, line 6,

> "The skilled addressee will appreciate that once the said at least two heterologous constructs are transformed into the moss plant cell, such as a moss protoplast, for example a *Physcomitrella patens* protoplast, which is then permitted to regenerate into moss protonema, for example of *Physcomitrella patens*, they will undergo recombination with each other many times over. This process, once initiated in the moss plant cell, increases the copy number of integrated transforming DNA constructs of the invention therein." (emphasis added)

Thus, the technical approach taken by the method provided in claim 1 includes amplifying gene expression in part by providing a system that directs intermolecular recombination. Again, recombination within the moss plant cell occurs between the constructs, which results in recombination many times over. Neither Gilbertson et al. nor Schaefer employ

the method of claim 1. Further, neither Gilbertson et al. nor Schaefer describe the technical approach taken by the present invention.

In claim 1, recombination occurs between constructs. After recombination the introduced hetereologous sequences are inserted into the nuclear genome of the moss plant cell. However, as discussed briefly above, Gilbertson et al. direct recombination between a construct and plastid DNA and not between constructs. That is, Gilbertson et al target recombination with the plastome. This is further emphasized at paragraph [0043], which provides,

> "As previously stated, plastid vectors are designed to target transgene integration into the plastid genome via homologous recombination. The location of transgene insertion must be chosen such that the insertion does not cause any disruption of normal plastid function. This is achieved by cloning the transgenes into a plastid intergenic region where no unidentified open reading frames exist, preferably such that readthrough transcription from the transgenes into neighboring resident plastid genes is avoided. Two plastid genomic locations are targeted for insertion of transgenes: the Large Single Copy region and the Inverted Repeat region. Because the Inverted Repeat region is present in two copies per genome, the transgenes will also be present in two copies in transformed lines."

Thus, whereas the present invention directs recombination between two constructs in a moss plant cell, which become integrated into the nuclear genome, Gilbertson et al. target recombination between a construct and plastid DNA.

In addition, the methods used for recombination also differ significantly. In claim 1, a first construct is flanked at the 5' end by a first recombination sequence and is flanked at the 3' end by a second recombination sequence, which is in the same orientation as the first; and a second construct is flanked at the 5' end by the second recombination sequence and is flanked at the 3' end by the first recombination sequence, which is in the same orientation as the second. The first and second recombination sequences are different and form a complementary set of recombination sequences enabling the constructs to recombine with each other.

In contrast, Gilbertson et al.'s method of inserting a transgene into the plastid DNA exploits a region of the plastid DNA that includes a repeating region, specifically TATTA repeat region. Thus, Gilbertson et al.'s approach is to flank the transgene in the construct with sequences that mimic the repeating region of the plastid DNA. Thus homologous recombination

selectively occurs at the regions of the plastid DNA having a TATTA repeat. Accordingly, central to Gilbertson et al is the development of a 5 base pair nucleic acid sequence motif or multiple repeats thereof. This is summarized in paragraph [0042], which provides in part,

> "More particularly, the regions of homology comprise the recombination sequence motif of the present invention. The recombination sequence motif comprises a 5 base pair nucleic acid sequence or multiple repeats thereof (whether in tandem or interspersed with other nucleotides) that increase the frequency of integration of a transgene. The recombination sequence motif generally comprises the sequence 5'-TATTA-3', its complement 3'-TAATA -5', or imperfect variations of such motif differing by a nucleotide[.]"

Although discussed above, an overview of the method is also shown in FIG. 5, which shows homologous recombination using the direct repeats 5'-TATTA-3' to target a TATTA repeating region. Thus, whereas claims 1-8 include first and second recombination sequences that are different and form a complementary set, and this complementary recombination set is further developed between constructs to enable recombination between constructs themselves; Gilbertson et al. direct homologous recombination between a construct and plastid DNA using a 5 base pair motif, which targets a repeat region in the plastid DNA. More specifically, Gilbertson et al. incorporate 5'-TATTA-3' motifs or repeats at each end of the transgene to target the TATTA repeat region in plastid DNA. Thus, the technical approaches themselves between claims 1-8 and Gilbertson et al. differ.

Since the present invention uses a significantly different technical approach than that taken by Gilbertson et al. the recombination method set forth by claims 1-8 is not obvious over Gilbertson et al.

With respect to Schaefer, Schaefer does discuss the transformation of the moss *Physcomitrella patens*. However, Schaefer does not discuss a system using a first heterologous nucleic acid construct flanked at the 5' end by a first recombination sequence and flanked at the 3' end by a second recombination sequence in the same orientation as the first; a second heterologous nucleic acid construct that is flanked at the 5' end by the second recombination sequence and flanked at the 3' end by the first recombination sequence in the same orientation as the second; and wherein the first and said second recombination sequences are different and form

a complementary set of recombination sequences enabling the constructs to recombine with each other.

Accordingly, claims 1-8 are not obvious over Gilbertson et al. in view of Schaefer. Thus, applicants respectfully request the rejection be withdrawn and the claims allowed.

Conclusion

In view of the amendments and arguments set forth above, applicants respectfully request the rejections be withdrawn and all claims allowed.

Respectfully submitted,

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